A STUDY OF TOXINS AND THE SEROLOGICAL REACTIONS IN SPRUE.

By Carl Michel, M.D.,

ASSISTANT SURGEON, U. S. PUBLIC HEALTH SERVICE, INSTITUTE OF TROPICAL MEDICINE AND HYGIENE OF PORTO RICO, SAN JUAN.

Ashford having shown the relationship of the Monilia psilosis to clinical sprue the necessity of studies of the biological activities of the Monilia psilosis as differentiated from other Monilia led to the use of various serological tests.

Very little being known about the disease-producing substances of the Monilia, experiments were made to determine the toxic substances of the Monilia and what substance could be used as a suitable constant antigen in the tests.

The Toxins. Cultures of virulent Monilia psilosis which had been isolated from fatal cases of sprue, and which, when inoculated (10 c.c.) into a guinea-pig killed it in forty-eight hours, were used. The culture was made on glucose-bouillon incubated forty-eight hours, then shaken in a machine for four hours, and filtered through a Berkefeld filter. From 2 to 10 c.c. of the filtrate were injected into several guinea-pigs. The animals at no time showed toxic symptoms or signs of infection. The same experiments were repeated with emulsions of cultures grown on solid media, and no symptoms of toxemia or infection appeared, which showed that no soluble toxin was present and that the toxin elaborated by Monilia psilosis is not extracellular. As a control, 5 c.c. of the unfiltered culture was injected into a guinea-pig, killing the animal in fifty-six hours. The Monilia was recovered from the heart, spleen, and liver. These experiments led to the study of the Monilia proteins and endotoxins.

Cultures of the Monilia being very difficult to kill, owing to the thermal death-point, which normally is 75° for ten minutes, and as that temperature is injurious to the antigenic enzymes, autolyzation was used as a means to lower the thermal death-point. Cultures of Monilia psilosis were autolyzed by suspending in distilled water, using one six-day culture to 10 c.c. of water. The emulsion was incubated for four days, after which 0.5 per cent. phenol was added and heated to 58° C. for forty-five minutes. Cultures made of the emulsion showed it to be sterile. The emulsion was filtered through a Berkefeld filter and 10 c.c. injected intraperitoneally into a guinea-pig. Slight toxic symptoms were observed from which the animal recovered within twenty-four hours. Five c.c. of the unfiltered emulsion injected intraperitoneally caused an animal to become severely sick with severe diarrhea and toxic symptoms, the animal losing 20 grams within two days. On the third day the symptoms subsided and the animal recovered.

An emulsion of Monilia psilosis was prepared, autolyzed, and killed, one four days slant culture growth being used for each 10 c.c. of 0.5 per cent. phenol solution. Animal and cultural tests were made to test its toxity and sterility. Fifteen persons, who gave no clinical symptoms of sprue and who in no way had been exposed to the disease, were injected. Each person was injected subcutaneously with 0.15 c.c. of the emulsion. A slight local reaction was noticed at the site of injection, which lasted about eighteen hours and then gradually disappeared, leaving a small indurated area. In one case there was a slight rise in temperature which dropped after forty-eight hours.

Several persons who had typical sprue were each injected with 0.1 c.c. of the emulsion. A pronounced reaction resulted and there was an aggravation of all the sprue symptoms. The reaction lasted for three or four days and slowly subsided. The local reaction persisted for five days. This showed that the Monilia proteins and endotoxins were to be considered as the active toxic substances in sprue, and would be of greatest antigenic value, and that these substances have the power to stimulate the production of antibodies in man.

AGGLUTINATION REACTION. To determine the specificity of the Monilia an agglutination test was used.

Two rows of tubes were set up, each containing 0.1 c.c. of normal salt solution. The serum of a clinically and culturally positive case of sprue was added in dilutions of 1 to 10, 1 to 5, and 1 to 2. To this was added 0.2 c.c. of a strong Monilia psilosis emulsion. In the other row normal serum was used as a control. The tubes were incubated eighteen hours and the results observed. In the tube which contained 1 to 2 dilution of positive serum a flaky precipitate was found upon slight agitation. The tubes containing the normal serum, upon agitation, remained uniform. Using this test as a basis a number of tests were made in which an emulsion of a Cryptococcus and normal serum were used for controls.

It was found that the agglutination test using the Monilia emulsion and known sprue serum was positive in only the cases which had severe sprue, with the serum of mild or latent cases the reaction was negative and no perceptible agglutination resulted.

Precipitin tests using Monilia filtrates were tried and did not yield any satisfactory results.

COMPLEMENT-FIXATION TEST. The complement-fixation test in sprue requires careful interpretation. In order to establish a positive standard, careful preliminary tests were made to determine the specificity of this test to the clinical cases. All of the tests made were checked up clinically and mycologically by Ashford.

PREPARATION OF ANTIGENS. Cultures of Monilia were made on Sabouraud's glucose media, incubated four weeks. The growth was washed off with distilled water, using one culture to 10 c.c.

The emulsion was shaken in a machine for two hours and incubated for four days to allow autolyzation. Half of 1 per cent. phenol was added as a preservative and the emulsion heated to 58° for one hour. Cultures were made as to its sterility. It was then titrated for its hemolytic and anticomplementary action. In the tests 1 c.c. was used as a standard. This emulsion has been used six months and the standard has not changed, which makes it a very stable antigen. Old cultures make the best antigens. Antigens made of young cultures lose their stability. Antigens made from the liver and heart of animals that died of acute sprue were of no value. Liver extracts from a patient dying of sprue gave unsatisfactory results.

Alcoholic Antigens. Emulsions of Monilia made in distilled water and incubated, after which the emulsion was heated to 58° for one hour and shaken in the machine for four hours. The emulsion was centrifuged and the supernating liquid poured off. To 1 c.c. of the sediment 15 c.c. of alcohol were added and incubated for two days. In the tests 1 c.c. of a 5 per cent. solution was used. The antigen showed positive in many cases, but was not constant in the delicate tests. The most preferable antigen is the watery emulsion, which is the most constant. The anticomplementary action of this emulsion is about 0.25 c.c. In the actual tests 0.07 to 1 c.c. was used.

Serum. Serum used was decomplementized by heating for an hour at 56° C., 2 c.c. of serum used. It is advisable to heat all serum to destroy the thermolabile anticomplementary enzymes. If natural only 1 c.c. was used. It is of interest to note that in Porto Rico the complement content of human blood was extremely high as compared to the United States, and heating at the 56° for thirty-minutes did not destroy all the complement. This was overcome by heating the blood at 56° for an hour and allowing it to stand for at least twelve hours before using.

Complement. In these tests 0.3 c.c. of a 10 per cent. solution of guinea-pig serum was used.

Amboceptor. Antisheep amboceptor $\frac{1}{1000}$ solution of serum titrated so that 1 unit=2 c.c. In the tests 1½ units or 0.3 c.c. were used.

Erythrocytes. 1 c.c. of a 5 per cent. solution of sheep corpuscles were used.

Technic. The technic employed in these tests were the Wassermann, Noguchi, and a modified Wassermann in which concentrated serums were used.

In the Wassermann test two series of tubes were set up. One series containing 0.1 c.c. of normal serum. In the other 0.2 c.c. of inactivated serum was used; 0.7 c.c. of Monilia psilosis antigen was added to each series. A control series of five tubes each was set up for each test, and the following antigens used; 0.1 c.c. of antigen prepared from a non-virulent Monilia isolated from feces, 0.1 c.c. of a white Monilia antigen, 0.1 c.c. of a red Cryptococcus antigen which was isolated from feces, 0.1 c.c. of a combined antigen prepared from mixture of all the antigens used. Another series of controls using syphilitic antigen was used. To all these tubes 0.3 c.c. of a 10 per cent. guinea-pig serum was added and 1 c.c. of salt solution. The tubes were then heated in a water-bath for half an hour, after which 0.3 c.c. of $\frac{1}{1000}$ antisheep amboceptor and 1 c.c. of 5 per cent. sheep erythrocytes was added. The tubes were again incubated for thirty minutes and the results taken.

In the Noguchi test similar technic was employed, using the same number of controls.

In the modified Wassermann test 1 drop of serum, 2 drops of antigen, 1 drop of undiluted complement and 10 drops of salt solution was placed in each tube. The tubes were then incubated for twenty minutes at 37° C., after which 2 drops of $\frac{1}{1000}$ sheep amboceptor and 0.2 c.c. of 10 per cent. sheep corpuscles were added. The tubes were again incubated for half an hour and the results taken. The Noguchi method proved to be the most delicate, and when this test was negative the cases were also found to be negative mycologically and clinically. In all cases which gave a positive result by the Wassermann reaction the Monilia psilosis was isolated from the feces and the case had clinical symptoms, with the exception of a few described in Ashford's article. The results of all these tests depend upon having proper antigens, and it is noted that antigens prepared from young cultures will not give a pronounced reaction.

SPECIFICITY OF THE TEST. Out of over 400 tests made, including various diseases, all cases which were diagnosed clinical sprue and from which the Monilia psilosis was isolated from the tongue and feces, the complement-fixation test was positive.

The other Monilia antigens used have faintly positive results only in severe cases of sprue. In these tests the results with other Monilia antigen was about 15 per cent. positive, while the reaction with the psilosis antigen was 100 per cent. positive. It is of interest to note that this reaction varies with the condition of the patient. In cases of chronic or latent sprue, and in cases which had recovered, the reaction tends to become negative.

Guinea-pigs which had been injected twice in ten days with 5 c.c. of a non-virulent Monilia psilosis culture gave positive tests, and as the animal recovered the reaction became negative.

Cases which had clinical syphilis and sprue, the fixation test for both these diseases was positive.

Pellagra having been demonstrated by Goldberger as a diet deficiency disease, the same view has been taken regarding sprue. In these series several cases of pellagra were included. All these cases were severe and had typical symptoms of that disease, and in no case was the Monilia psilosis isolated. The complement-fixation test in all these cases was negative, with the exception of one faintly positive.

Guinea-pig serum of an animal which had been inoculated with a killed culture of Monilia psilosis gave a strong positive reaction.

It is of interest to note that the serum from animals which had been inoculated with live cultures or killed cultures of Monilia psilosis gave the same results as the serum of patients that have sprue, and from which the Monilia psilosis had been isolated from tongue and feces. In view of these results and with Ashford's clinical and mycological work it is strongly evident that the Monilia psilosis of Ashford is the etiological factor in sprue. At the present time tests are being made daily in this laboratory, with the same constant results as described in this article.

THE DISINFECTION OF DRINKING WATER.

By H. D. Dakin, D.Sc., F.R.S.,
NEW YORK,
AND
E. K. Dunham, M.D.,
NEW YORK.

(From the Herter Laboratory, New York.)

The sterilization of contaminated drinking water is a problem of special importance in war time. The use of bleaching powder, or similar hypochlorite or chlorin preparation, has been used with the greatest success for the sterilization of relatively large volumes of water. The use of water carts containing water treated with a carefully regulated quantity of bleaching powder has given every satisfaction when the method could be satisfactorily carried out, as, for example, when troops are practically stationary. The problem of sterilizing small individual quantities of water, such as are needed by cavalry or rapidly moving troops, is a much more difficult problem which, up to the present, has not been perfectly solved. The use of hypochlorites for such purposes is difficult, owing to the instability of small tablets containing the minute quantity of active disinfectant. In their place, acid sulphates of the alkali metals have been extensively used in tablet form, but the superior potency of many chlorin compounds would indicate that a stable potable chlorin disinfectant suitable for the sterilization of small quantities of water was desirable. With this end in view we have made a number of experiments with various types of substances, one of which we believe to be worth practical application.